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Influence of the electric field on a bio-mimetic film supported on a gold electrode

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Abstract

A model biological membrane was formed by fusion of mixed cholesterol and DMPC (dimyristoylphosphatidylcholine) phospholipid vesicles onto a gold-coated quartz support. The gold surface was charged and the influence of the charge at the solid support on the structure and integrity of the phospholipid bilayer was investigated using the specular reflection of neutrons and electrochemical measurements. When the surface charge density is close to zero, the lipid vesicles fuse directly on the surface to form a bilayer with a small number of defects and hence low water content. When the support's surface is negatively charged the film swells and incorporates water due to the field driven poration of the membrane. When the charge density is more negative then $-8\,\mu C\,cm^{-2}$ the bilayer is detached from the metal surface. However, it remains in close proximity to the metal electrode, suspended on a thin cushion of water. The film thicknesses, calculated from neutron reflectivity, have allowed us to determine the tilt angle of the lipid molecules as a function of the support's charge density. The lipid molecules are tilted 55° from the surface normal at zero charge density but become significantly more perpendicular (30° tilt angle) at charge densities more negative than $-8\,\mu C\,cm^{-2}$. The tilt angle measurements are in very good agreement with previous IR studies. This paper describes the highlights of a more in-depth study which is fully described in [1].
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Keywords: Biological membrane; Phospholipid vesicles; Quartz

1. Introduction

Phospholipid bilayers formed by fusion of small unilamellar vesicles (SUVs) onto glass or organic-modified surfaces constitute an attractive model of a biological membrane to study membrane processes [2–5]. Monolayers [6,7] and bilayers [8–10] of phospholipids may also be deposited or tethered to surfaces of metal electrodes such as mercury or gold. They may then be used to study the influence of electric field on voltage-gated membrane proteins and lipid–lipid and lipid–protein interactions [11–13]. In addition, films of phospholipids with incorporated proteins deposited at a metal or metal oxide electrode find important application in the development of electrochemical sensors [14]. At an electrode surface, phospholipid films are exposed to high electric fields on the order of 10^9 V/m. A field of this magnitude affects the membrane properties and knowledge of this effect is needed for both scientific and practical applications of supported phospholipid bilayers.

2. Experimental

2.1. Preparation of vesicles

Vesicles were prepared by the method described in [15]. Solutions of DMPC (Avanti Polar Lipids, Birmingham, AL) and cholesterol (>99%, Aldrich) in chloroform (>99.9%, Aldrich) were combined in a test tube to form a 7:3 (DMPC:cholesterol) mole fraction mixture. The solvent was evaporated to dryness by vortexing the mixture under a stream

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of argon. Complete removal of the chloroform was achieved by placing the test tube in a vacuum desiccator for a minimum of 2 h. The DMPC:cholesterol mixture was stored, under vacuum, in this state until the commencement of the NR and electrochemical experiments. A sufficient volume of electrolyte solution was then added to the tube to give a 1–2 mg/mL solution. Vesicles were formed by sonicating the solution for 120 min at 40 °C.

2.1.1. Electrochemistry

The method used to determine the charge density has been described elsewhere [16,17].

2.1.2. Neutron reflectivity

Details concerning the acquisition of specular reflected neutrons in an electrochemical environment have been described elsewhere [18,19].

To extract structural information from the reflectivity data the reflectivities were analyzed using an iterative, dynamic procedure [18–20]. Based on known facts about the system (the thickness of the gold and chromium layers and the anticipated structure of the organic layer at the interface) a model SLD profile was generated and used to calculate the reflectivity curve. The calculated reflectivities were then compared with the experimental data. A least squares iterative procedure was then used to vary the parameters of the SLD profile until a good fit between the calculated curve and the experimental data was achieved, using the Parratt 32 fitting algorithm [21].

3. Results and discussion

We have performed electrochemical and neutron reflectivity studies on the influence of the electric field on the behavior of a mixed dimyristoylphosphatidylcholine (DMPC)/cholesterol bilayer (70:30 mol% ratio), deposited on a Au(1 1 1) electrode surface by direct vesicle fusion. To facilitate the spreading of the vesicles, the measurements were performed at 30 °C where the membrane is in the liquid-crystalline state. DMPC is a neutral (zwitterionic) lipid and hence this membrane is uncharged. The field acting on this membrane is solely due to the charge on the metal. We employed electrochemical methods to measure the charge density on the electrode surface σ_M [10] and then calculated the field acting on the membrane using Gauss' theorem ($F = (d\varphi/dx)_{x=0} = \sigma_M/\varepsilon$), where $\varepsilon = n^2\varepsilon_0$, $n = 1.45$ is the refractive index of DMPC and $\varepsilon_0 = 8.85 \times 10^{-12}$ C V^{-1} m^{-1} the permittivity of vacuum.

Fig. 1 plots the charge at the Au electrode for the bilayer-free (background) and the bilayer-coated surface as a function of the applied potential. The electrode potential is the operational variable that is controlled by an instrument, while charge is the physical variable that measures the field acting on the membrane. The membrane is stable at the electrode surface in the charge density range $\pm 8\,\mu C\,cm^{-2}$, where the

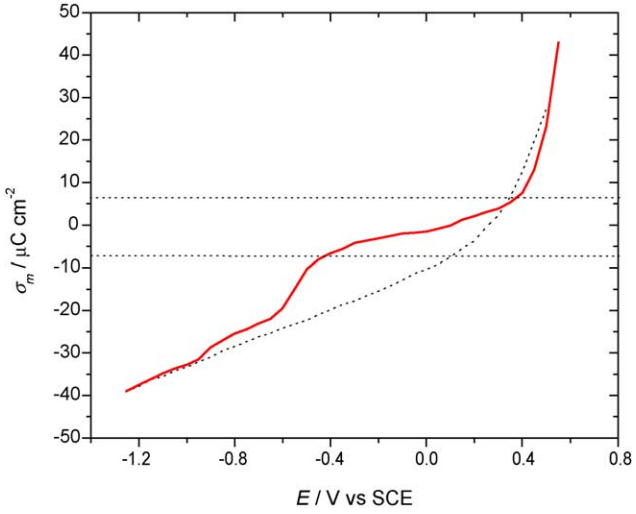

Fig. 1. The measured surface charge density on the gold electrode surface plotted vs. the electrode potential which is the experimentally controlled variable for the pure background 50 mM NaF supporting electrolyte (dotted line) and a mixed cholesterol:DMPC bilayer spread from a vesicle solution (full line). The horizontal dotted lines represent the range of surface charge densities found on naturally occurring lipid bilayers.

charge changes slowly with potential. Above or below these values the charge density curve for the membrane-covered electrode rises or falls quickly to merge with the curve for the film-free interface. This behavior indicates that the membrane is detached from the gold surface. A charge density of $8\,\mu C\,cm^{-2}$ corresponds to a field of $\sim 5 \times 10^9$ V/m. The membrane is therefore stable when exposed to fields less than the above value.

To appreciate the significance of these numbers it is instructive to compare the charge on the metal with the charge present at the surface of a natural biological membrane. A natural membrane has between 10 and 20 mol% of anionic phospholipids and this corresponds to one negative charge per 300 Å^2 of the membrane surface [22] or $\sim -5\,\mu C\,cm^{-2}$. The charge densities on the surface of a natural membrane and the charge acting on a neutral model membrane deposited at the Au electrode surface are therefore comparable. The applied field at the supported membrane closely mimics the field acting on a natural membrane, which means that such membranes supported on electrode surfaces make good models to study field-driven membrane processes. The uniqueness of our experiment was that the direction and the magnitude of the field could be varied continuously. We were therefore able to study how the membrane responds to the broad range of electric fields acting on the membrane.

We have performed neutron reflectivity experiments to investigate the field driven changes of the membrane structure. Neutron reflectivity measurements have been employed previously to study supported bilayers deposited at a quartz surface [22–25]. The intensity of neutrons reflected from the interface provides information on the scattering-length density (SLD) profile in the direction normal to the surface. The SLD depends on the number

density of the scatterer and hence the SLD profile maps the density of scatterers in the direction normal to the surface. The SLD for gold, hydrogen and deuterium are distinctly different and a strong contrast is achieved when a film of non-deuterated phospholipids is deposited at a gold electrode surface from a solution of D_2O. In that case, the thickness of the bilayer and the water content in the bilayer may be determined from SLD curves. Here, we report the first neutron reflectivity studies of a model membrane exposed to high electric fields and we demonstrate the uniqueness of this technique to determine the field driven changes of membrane structure.

To mimic the charge acting on a natural membrane, the experiments were carried out at a negatively charged surface. The charge density was varied gradually from 1 to $-27\,\mu C\,cm^{-2}$. Fig. 2A shows the reflectivity curve for the membrane formed at the gold electrode at charge density $\sim 1\,\mu C\,cm^{-2}$. This reflectivity curve gives a good fit to the SLD profile shown as a red line in Fig. 2B, assuming a single layer of non-deuterated molecules deposited directly on the metal surface. This fit requires only two adjustable parameters; however, it averages the SLDs for the head and tail regions of the phospholipids. In order to extract more detailed information on the membrane, the same reflectivity data was fit to a three-layer model assuming that the membrane consists of two 8 Å thick polar head regions and a middle section composed of non-deuterated acyl chains. While the three-layer model is physically more realistic and statistically gives a slightly better fit to the experimental data, it involves five adjustable parameters. The contrast for the polar head region and the backing gold or D_2O phases is weak. For that reason, the SLD values for the two head-group layers are not certain (green line in Fig.2B). However, we were concerned chiefly with the SLD profile in the middle layer that gives a strong contrast with the electrode and the solvent. The thickness and the SLD values for the acyl chain region of the membrane are compiled in Table 1.

The coherence length of neutrons is on the order of 10–100 μm and hence the reflectivity experiment averages the SLD values in the layer parallel to the interface over that range of dimensions. The SLD data may therefore be used to calculate the volume fraction of water present in the middle section due to the imperfections in the membrane formed by the fusion of SUVs. These numbers are also compiled in Table 1. Similar reflectivity curves were recorded when the negative charge on the metal was progressively increased. The one layer (and the more physically correct three-layer) model of the membrane deposited directly onto the gold surface fit the reflectivity curves well in the charge density range from 1 to $-8\,\mu C\,cm^{-2}$. Qualitatively, the SLD profiles resembled the curves shown in Fig. 2B. However, the data in Table 1 show that the thickness and the water content in the membrane increase with the charge on the metal. In the presence of a high electric field, the membrane swells and becomes more porous.

The results for $\sigma_M < -8\,\mu C\,cm^{-2}$ were surprising. Fig. 1 shows that charge for the surface that was initially film-covered drops and eventually becomes equal to the charge for the film-free interface. This behavior indicates that the film is detached from the metal surface. We expected that at highly negative surface charge densities the reflectivity curve would also merge with the curve for the film free-electrode. In contrast, at $\sigma_M = -23\,\mu C\,cm^{-2}$, Fig. 3A shows that the reflectivity of the surface initially covered by the film remains much higher than the reflectivity of the film-free interface. Apparently, the film remains in close proximity to the surface. In fact, this experimental data can be fit by allowing for a ~8 Å thick layer of D_2O to separate the membrane from the gold electrode surface. The SLD profile fitting the reflectivity curve is shown in Fig. 3B. The red line plots the profile for the membrane represented as a one layer model and the green line the profile for the three-layer model of the membrane. One can note that differences between the membrane structure at $\sigma_M \sim 1\,\mu C\,cm^{-2}$ and at $\sigma_M = -23\,\mu C\,cm^{-2}$ are significant. At high negative charges, the middle section of the membrane is much thicker and is essentially solvent (i.e. defect) free. In fact, at high negative charges, the membrane structure resembles the structure of a DMPC bilayer supported on a quartz surface [23,25], where a 10–20 Å thick layer of water also separates the phospholipids from the surface of the solid support. A quartz surface is negatively charged when in contact with an aqueous solution, having a charge density of $\sim -20\,\mu C\,cm^{-2}$ when the solution pH is approximately 8 [26]. It is therefore not surprising that the properties of the

Table 1
Values obtained from the fitting of the neutron reflectivity data for the cholesterol:DMPC bilayer supported on the gold electrode surface at different surface charge densities

σ_m ($\mu C\,cm^{-2}$)	D_2O layer		Tail region		
	τ (Å)	ρ ($\times 10^6$ Å^{-2})	τ (Å)	ρ ($\times 10^6$ Å^{-2})	f
1.2	N/A	N/A	19.0	0.3	0.08
−3.9	N/A	N/A	20.5	0.8	0.15
−6.6	N/A	N/A	23.6	1.2	0.22
−10.4	2.1	4.9	26.8	1.1	0.20
−19.4	5.7	5.1	27.7	1.1	0.20
−23.0	6.8	5.7	29.1	−0.2	0
−27.0	8.0	6.0	29.3	−0.2	0

τ is the thickness, ρ is the scattering light density, f is the volume fraction of D_2O in the bilayer.

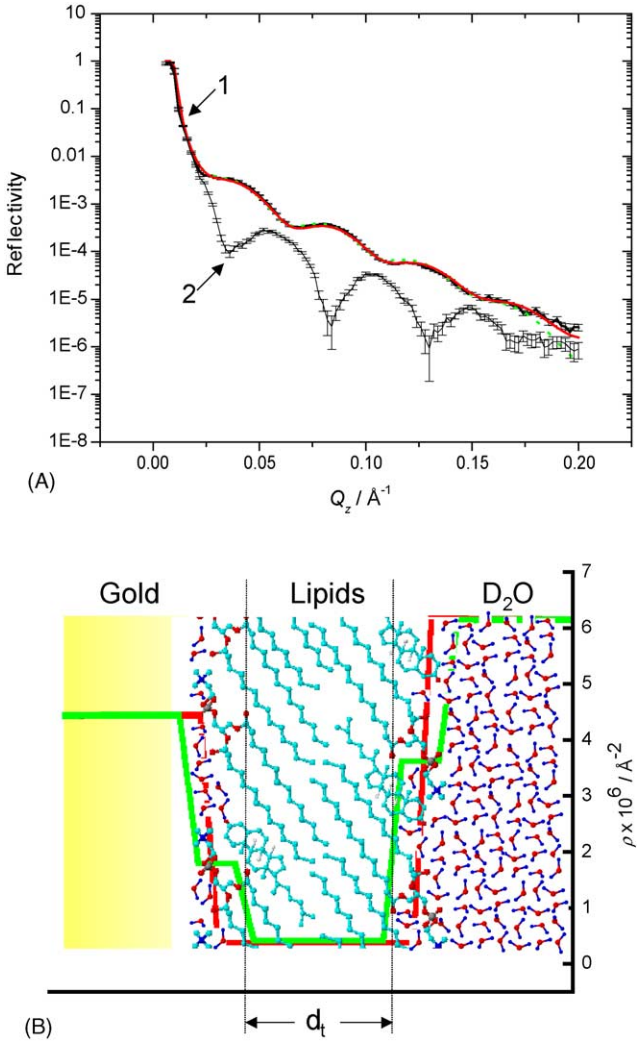

Fig. 2. (Part A) Shows the neutron reflectivity data (curve 1) obtained for the a mixed cholesterol:DMPC bilayer spread at the electrode surface when the surface charge density of the gold is very low, i.e. 1.2 μC cm^{-2}. The red line is the resultant fit from a one box model used to represent the adsorbed film whereas the green dotted line results from a three box model which divides the film into two headgroup layers and one acyl tail layer. See text for further discussion. Curve 2 is a background reflectivity curve measured for the electrode in the absence of the biological film. (Part B) Displays a real space profile of the bio-mimetic film/gold interface in the direction perpendicular to the electrode. The red and green lines are the scattering length density (SLD) profiles, determined from the neutron reflectivity curve given above in Part A, for the one and three-layer models, respectively. The model shows hydrated headgroups directly adsorbed on the electrode surface.

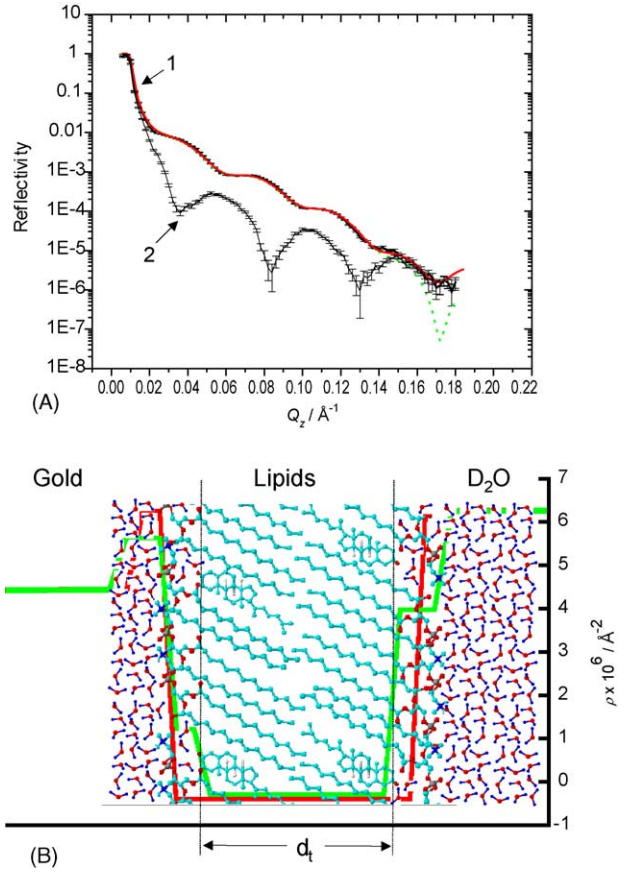

Fig. 3. (Part A) Shows the neutron reflectivity data (curve 1) obtained for the a mixed cholesterol:DMPC bilayer spread at the electrode surface when the surface charge density of the gold is highly negative, i.e. −23.0 μC cm^{-2}. The red line is the resultant fit from a one layer model used to represent the adsorbed film whereas the green dotted line results from a three-layer model which divides the film into two headgroup layers and one acyl tail layer. Curve 2 is a background reflectivity curve measured for the electrode in the absence of the biological film. By comparing curves 1 and 2 it is important to note that even though the film is now no longer directly attached to the gold electrode (see text and Part B below), neutron reflectivity still detects the biological film in close vicinity to the support. This is a unique ability of the neutron reflectivity technique. (Part B) Displays a real space profile of the bio-mimetic film/gold interface in the direction perpendicular to the electrode. The red and green lines are the scattering length density (SLD) profiles, determined from the neutron reflectivity curve given above in Part A, for the one and three box models, respectively. The model shows a defect-free biological film with no solvent penetration. In contrast to Fig. 2B, the headgroups of the lipid molecules are no longer directly adsorbed on the electrode surface but rather supported by a cushion of solvent molecules.

membrane supported at a negatively charged gold surface are similar to those of a membrane supported on a quartz surface.

The fact that the charge–potential curve for the membrane coated surface merges with the curve for the membrane free interface at high negative charges indicates that the capacity of the electrode with the membrane supported on a cushion of the solvent and the membrane free electrode have comparable magnitude. This behavior indicates that the layer of solvent that separates the membrane from the metal contains ions of the electrolyte, which effectively screen the charge on the metal. Despite the fact that the charge on the metal is high, the field acting on the membrane is weak. In reality, both at charge 1 μC cm^{-2} and at very negative charges, the field acting on the membrane is weak. The dramatic difference between the membrane structure at $\sigma_M \sim 1$ and $\sigma_M = -25$ μC cm^{-2}, shown in Figs. 2 and 3, is due chiefly to the presence or absence of a D_2O layer at the gold surface. Charging of the metal surface affects its hydrophilicity. At small charge den-

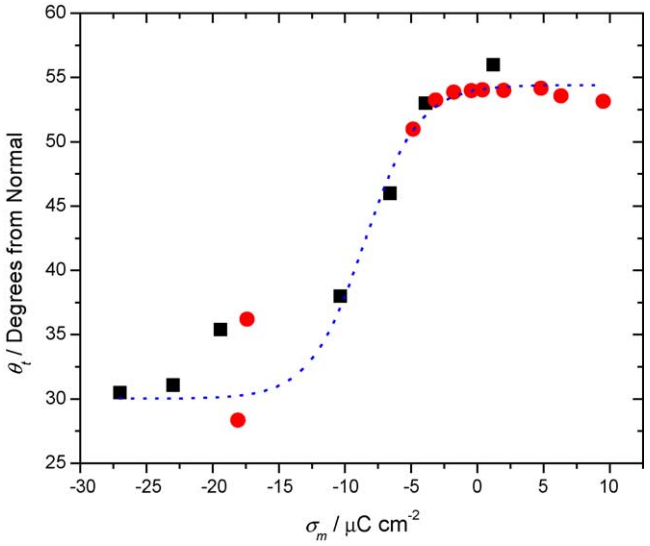

Fig. 4. Acyl tail of DMPC tilt angle as a function of the surface charge density on the gold electrode support determined from infrared spectroscopy (●) and from the present neutron reflectivity study (■). The dotted line is shown to guide the eye.

sities a gold surface is hydrophobic whereas at high charges the surface is hydrophilic. These results demonstrate that the membrane has a different structure when supported on a hydrophobic and a hydrophilic surface.

The data in Table 1 show that the thickness of the tail section of the membrane significantly changes with the charge on the metal. This behavior indicates that the chain tilt changes with charge. The maximum length of the hydrocarbon tail for DMPC is $d_{max} = 16.7$ Å. This number may be used to calculate the chain tilt angle θ with the help of the formula $\cos\theta = d_T/d_{max}$. The chain tilt angle is plotted against the charge in Fig. 4. For comparison the chain tilt angles for a pure DMPC membrane at a Au(1 1 1) electrode, determined recently by infrared reflection spectroscopy [10] are also plotted in this figure. The agreement between the neutron reflectivity and IR data is very good. The changes in the tilt angle with charge are striking. When the membrane is deposited directly at the metal the chains are tilted at ∼55° with respect to the surface normal. At high negative charges when the membrane is lifted from the metal and is suspended on a cushion of D_2O, the tilt angle decreases to 30°. Incidentally, the latter number agrees well with the chain tilt angle in a DMPC lamellae measured by X-ray diffraction [27] and with the chain tilt angle in a DMPC bilayer supported on quartz determined by neutron reflectivity [23].

4. Concluding remarks

We have demonstrated that a model biological membrane supported at a metal surface is stable when the charge on the metal is less than $-8\,\mu C\,cm^{-2}$ or the field acting at the membrane is less than 5×10^9 V/m. At charges near zero on the metal the acyl chains are tilted at a large angle with respect to the surface normal. The membrane is thin and contains defects. By charging the metal and increasing the field, the chains become less tilted. The membrane swells. It is thicker and more porous. When $\sigma_M < -8\,\mu C\,cm^{-2}$ and the field exceeds 5×10^9 V/m the membrane is detached from the electrode. We have shown for the first time that this membrane remains in close proximity to the metal electrode, being suspended on a thin cushion of the electrolyte. This membrane is essentially defect-free. The charge-driven changes in the membrane structure are fully reversible. By turning a knob on a control instrument one can inject or withdraw the charge from the metal surface. The membrane responds to these changes by either lifting up or depositing directly on the metal. This ability to use the electric field to control the membrane structure opens new opportunities for bio-mimetic research.

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